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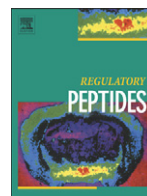
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The effect of ileal interposition surgery on enteroendocrine cell numbers in the UC Davis type 2 diabetes mellitus rat



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ABSTRACT

Aim: To investigate the short-term effect of ileal interposition (IT) surgery on gut morphology and enteroendocrine cell numbers in the pre-diabetic UC Davis type 2 diabetes mellitus (UCD-T2DM) rat.

Study design: Two-month old male UCD-T2DM rats underwent either sham (n = 5) or IT (n = 5) surgery. Intestines were collected 1.5 months after surgery. The jejunum, ileum and colon regions were processed for histochemical and immunohistochemical labeling and stereological analyses of changes in gut morphometry and number of enteroendocrine cells.

Results: Stereological analysis showed that intestinal volume, luminal surface area and the number of all chromogranin A-positive enteroendocrine cells were markedly increased in the IT rats compared with sham-operated animals. Subanalyses of the glucagon-like peptide 2, cholecystokinin, serotonin cells and the neurotensin immunoreactive sub-pool of enteroendocrine cells in the IT region revealed an increase in numbers across phenotypes. However, the density of the different cell types varied.

Conclusion: IT surgery in the UCD-T2DM rat leads to rapid alterations in gut morphometry and an increase in the number of enteroendocrine cells. This effect may potentially explain why IT surgery delays the onset of type 2 diabetes in the UCD-T2DM rat.

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1. Introduction

The marked effect of bariatric surgery and gut resection on intestinal morphology has been a matter of intense investigation for more than half a century [1,2]. The numerous intestinal adaptations include significant alterations in crypt cell proliferation, lengthening of the villi, increases in the crypts-to-villi ratio and an overall increase in the mucosal weight [3]. The responsible factors may be diverse. Peptides, growth factors, cytokines, blood flow and neural influences have all been suggested to play important roles in causing these changes in the intestine [4–7].

Recently, much attention has been paid to gastrointestinal (GI) peptides such as glucagon-like-peptide-2 (GLP-2) which function as a trophic gut hormone [8,9]. The intestinotrophic effects of GLP-2 have led to the development and recent approval of a GLP-2 analog for the treatment of short bowel syndrome [10,11]. GLP-2 is co-secreted with both GLP-1 and oxyntomodulin from the processing of proglucagon in the intestinal L-cells [12,13] distributed throughout the intestinal

tracts with the highest numerical density being in the distal ileum [14,15]. In addition to proglucagon, L-cells are also known to express peptide tyrosine tyrosine (PYY) which leads to a marked inhibition of food intake in rodents and man [16,17]. Recently, we demonstrated that Roux-en-Y gastric bypass (RYGB) in Wistar rats is coupled with an increased number of intestinal L-cells, as well as increased preproglucagon and PYY mRNA expression, thereby providing a plausible explanation for the powerful effect of this kind of surgery on body weight and resolution of type 2 diabetes [18]. Elevated post-prandial levels of GLP-1 and PYY are also a general trait of ileal interposition (IT) surgery [19] with associated effects on glucose homeostasis [19–22]. However, relatively little is known about this kind of surgery on endocrine cell numbers.

Due to their important clinical application for the treatment of type 2 diabetes and potentially also body weight [23,24] L-cell derived peptides has received increased focus. However, a number of other enteroendocrine (EEC) cell types have important roles in digestive physiology and obesity related diseases (for review, see [25]). It is therefore of potential importance to understand the quantitative and qualitative alterations in those cell types as well to facilitate our understanding of the metabolic benefits and the implications of bariatric surgery. EECs have traditionally been divided into different subtypes

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based on their hormonal content [26,27] but although their secretory phenotype may vary, they also share a number of common morphological characteristics. This includes the presence of secretory vesicles, components of which can be exploited as general marker for EECs [28]. Double immunohistochemistry against chromogranin A, a matrix-soluble glycoprotein commonly found in secretory vesicles [28], and a number of different peptidergic hormones have demonstrated that chromogranin A co-localizes not only with serotonin (5-HT positive cells), but also with cholecystokinin, neurotensin and PYY/GLP-1 positive L-cells in the GI tract, thereby supporting the use of chromogranin A as a general marker for EECs [29].

In this study we aimed to examine changes in intestinal volume, intestinal surface area and the total number of chromogranin A immunoreactive EECs, as well as specific subtypes, following IT surgery in the pre-diabetic UCD-T2DM rat model [30,31]. In this animal model, IT surgery has been shown to improve glucose and lipid metabolism and delay the onset of diabetes potentially associated with increased GLP-1 and PYY secretion, increased circulating bile acid concentrations, decreased endoplasmic reticulum (ER) stress signaling and improved beta-cell function [31].

However, the effect of IT surgery on gut morphology and EEC numbers has not previously been assessed using stereological methods.

2. Materials and methods

2.1. Animals

The present study is based on tissues from a published *in vivo* study [31]. Animals were housed individually in hanging wire cages in the animal facility at the Department of Nutrition at the University of California, Davis (UCD), USA and maintained on a 14:10-hour light–dark cycle. At two months of age rats underwent sham ($n = 5$) or IT surgery ($n = 5$) and were terminated 1.5 months later for tissue collection. All animals received ground chow (no. 5012; Ralston Purina, Belmont, CA, USA). Food intake and body weight were recorded three times a week. Diabetes onset was monitored by measuring non-fasting blood glucose weekly with a glucose meter (One-Touch Ultra, LifeScan, Milpitas, CA, USA) at 14:00–16:00 h. Diabetes onset was defined as a non-fasted blood glucose value above 11.1 mmol/l (200 mg/dl) for two consecutive weeks. The experimental protocols were approved by the University of California Davis Institutional Animal Care and Use Committee.

2.2. Oral glucose tolerance test

An oral glucose tolerance test (OGTT) was performed in all animals at 1 month after surgery. Animals were fasted overnight (12 h) and then received a 50% dextrose solution (1 g/kg BW) by oral gavage. Blood was collected from the tail for measurement of glucose and insulin concentrations. A second aliquot of blood was placed in tubes containing ethylenediaminetetraacetic acid (EDTA), aprotinin and a dipeptidyl peptidase-4 inhibitor and analyzed for total GLP-1 and active GLP-1.

2.3. Hormone and metabolite measurements

Fasting (13 h) EDTA plasma and whole blood samples were collected at baseline and on the day of euthanasia. Plasma was assayed for glucose using enzymatic colorimetric assays (Thermo DMA) and insulin using ELISA (Millipore). Total GLP-1 was measured by sandwich electrochemiluminescence immunoassay (Meso Scale Discovery). HbA_{1c} was measured using an enzymatic colorimetric assay (Diazyme; Poway, CA, USA). Serum glucose was measured using an enzymatic colorimetric assay for glucose (Thermo DMA Louisville, CO, USA). Insulin and total GLP-1 were measured by sandwich electrochemiluminescence immunoassay (Meso Scale Discovery;

Gaithersburg, MA, USA). Plasma GLP-1_{7–36} was measured by rodent/rat specific ELISAs (Millipore, St. Charles, MO, USA).

2.4. IT surgery

IT surgery was performed according to the procedure described by Koopmans and Sclafani [20]. Rats were placed on a liquid diet (Boost; Novartis, Minneapolis, MN, USA) for 4 days prior to surgery and 7 days post-surgery and received enrofloxacin (20 mg/kg subcutaneously) before and after surgery. Anesthesia was induced and maintained with isoflurane (2%–5%). A midline abdominal incision was made and a 10-cm segment of ileum 5–10 cm proximal to the ileocaecal valve was isolated and transected. An anastomosis was formed with the remaining ends of the ileum using 7-0 PDS suture (Ethicon®). Next, a transection was made 5–10 cm distal to the ligament of Treitz and the isolated ileal segment was inserted isoperistaltically. The transposed segment remained innervated with its vasculature intact. Sham surgeries were performed by making transections in the same locations as in the IT-operated animals.

2.5. Tissue sampling

At termination, the intestinal tract was removed, fixed by immersion in 4% phosphate buffered formaldehyde and stored at 5 °C until further processing. The jejunioileum, defined from the distal end of the duodenum to the anterior part of the cecum, was carefully dissected and subdivided into four distinct segments based on surgical scars from the site of anastomosis (Fig. 1 A). The colon was included as a separate fifth segment, while the duodenum was excluded due to tissue damage during pancreas removal [31]. Each segment was carefully measured, pinned down and embedded in a 4% agarose gel after which 8–12 biopsies were sampled systematically uniform randomly across each segment. The agarose slabs were infiltrated overnight in paraffin, mounted two and two together in blocks of paraffin and eventually cut into 5 µm thick paraffin sections on a Microm HM340E (Thermo Scientific, Florida, USA). Sections were mounted individually on separate object glasses, or pair-wise as two consecutive sections.

2.6. Immunohistochemistry

The current antibody has previously been shown to co-localize 100% with GLP-1 in brainstem preproglucagon expressing neurons [32]. EECs were identified by immunohistochemistry using the following procedure: sections from the IT region were deparaffinized in toluene and rehydrated in series of ethanol to water. Sections were then subjected to antigen retrieval in Tris-EGTA buffer, blocked for endogenous peroxidase activity and non-specific binding before being incubated with the following primary antibodies for 1 h: Chromogranin A diluted 1:100 (ab45179, AbCam, Cambridge), neurotensin diluted 1:3200 (ab43836, AbCam, Cambridge), 5-HT diluted 1:1600 (rabbit polyclonal antibody, Gubra, Denmark), CCK diluted 1:3200 (rabbit polyclonal antibody, Gubra, Denmark), GLP-2 diluted 1:16,000 (GLP2-12 F21-A7 antibody, Novo Nordisk, Denmark). Sections were subsequently visualized using Envision (K4007 and K4010, DAKO, Glostrup, Denmark) and finally developed using DAB (DAKO, Glostrup, Denmark) as chromogen. Slides were counter-stained with hematoxylin, dehydrated, coverslipped with Pertex (Sakura, Copenhagen, Denmark) and finally digitalized on an AperioScanscope AT slide scanner (Aperio, California, USA).

2.7. Stereological estimation of volume

The GI volumes were estimated using newCAST (Visiopharm, Hørsholm, Denmark) on digital slides. On each section fields of views were sampled in a random systematic way using the newCAST software. The volumes of the mucosal layer, as well as the submucosa/muscularis/

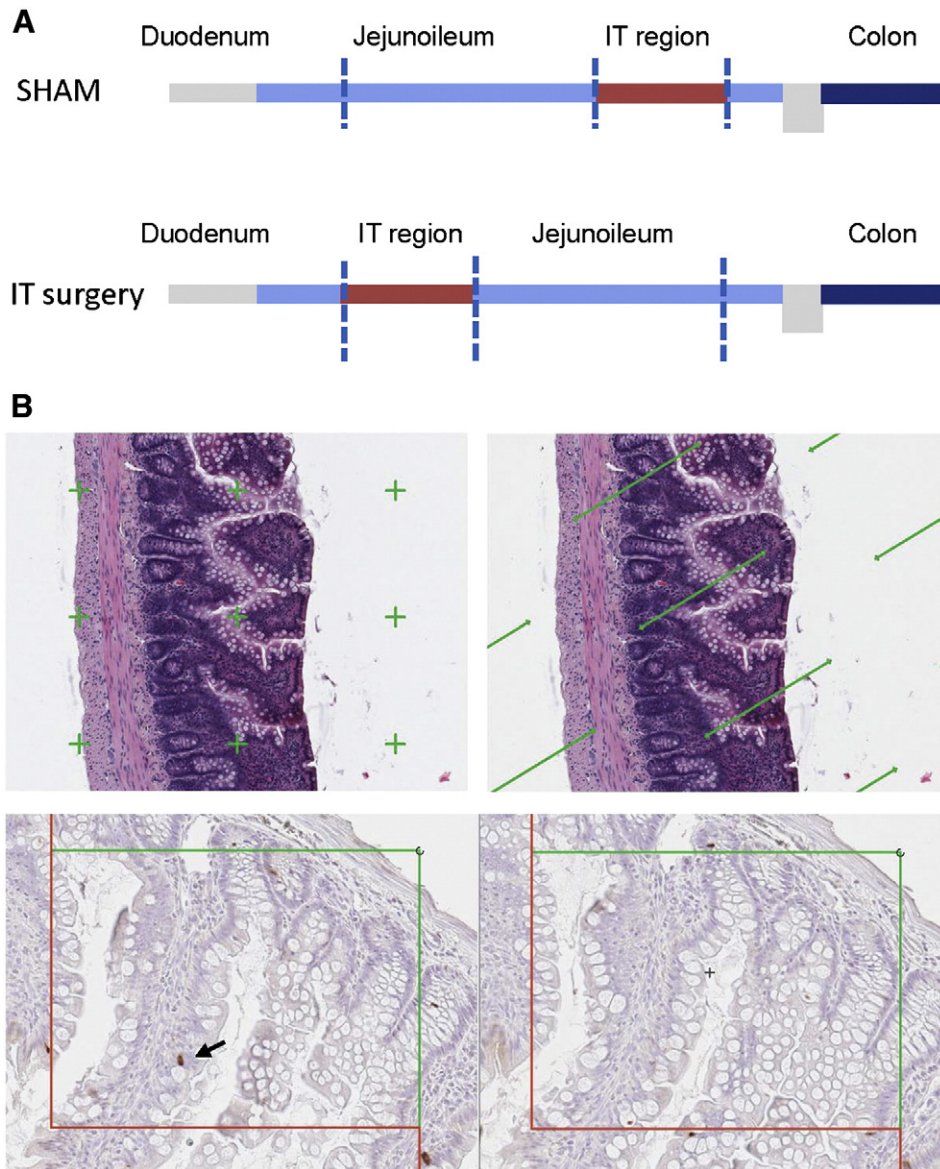


Fig. 1. Tissue sampling and stereological procedures: graphical illustration of the ileal transposition procedure in sham and IT surgery groups. The whole jejunum was dissected and divided into anterior jejunum, IT region, jejunum and distal ileum based on surgical scars (A). Regional and specific cellular layer volume (i.e. the mucosa, submucosa and muscularis) was estimated by point counting (B – top left). Surface area was estimated by counting intersections between line probes and the mucosal surface (B – top right), and number was estimated using the physical disector on two paired neighbor sections (B bottom panel). Arrow indicates the counting event where an immunoreactive cell is evident in one section and not the other.

serosa layer, were estimated using a 16-point grid at 10× magnification (Fig. 1B). The number of points hitting the structure of interest was converted into volume using the principle of Cavalieri:

$$\text{Vol}_{\text{ref}} = \sum p \cdot A(p) \cdot t$$

where $\sum p$ is the total number of points hitting the structure of interest, $A(p)$ is the area associated with each grid point and t was the distance between sections [33].

A shrinkage coefficient was used to convert processed shrunken tissue into the total original volume and surface area. In-section shrinkage induced by dehydration and paraffin embedding was estimated by comparing point counts performed on frozen unshrunk sections with paraffin-embedded sections. Shrinkage along the longitudinal

axis was estimated by subjecting 20 mm long agarose embedded segments to dehydration before re-measuring the length.

2.8. Stereological estimation of intraluminal surface area

The intraluminal surface density and area were estimated by counting intersections between linear probes and the luminal side of the gut [34] (Fig. 1B). Surface density (S_v) was estimated by the relationship between intersection and point counts using the formula:

$$S_v = \frac{2 \cdot \sum I}{l_p \cdot \sum P}$$

where $\sum I$ was the number of intersections of the test lines with the epithelium of the tunica mucosa, l_p was the test line length associated

with a point of the grid and $\sum P$ was the number of test points hitting the reference volume. Finally, the absolute surface area was estimated by multiplying the surface density with the reference volume using the formula:

$$S = S_v \cdot \text{Vol}_{\text{ref}}$$

A linear shrinkage factor was assessed to correct surface shrinkage [35].

2.9. Estimation of the total number of enteroendocrine cells

The total number of immunoreactive cells in the IT region (see Fig. 1) was estimated using the principle of the physical dissector [34,36]. For this purpose tissue sections were sampled as two consecutive 5 μm sections, thus obtaining two adjacent sections on one slide. The slides were processed for immunoreactivity, digitized and finally analyzed on a computer running newCAST software at $\times 20$ magnification. The total number of stained cells in a defined sampling volume was counted and the particle density N_v was calculated as:

$$N_v = \frac{\sum Q}{a(\text{frame})} \cdot h \cdot \sum P$$

where $\sum Q$ is the total number of uniquely counted cells, $a(\text{frame})$ was the area of the counting frame, h was the distance between the two sections, and $\sum P$ was the total number of points hitting the reference space. The total number of EECs was finally determined by multiplying N_v with the total reference volume.

2.10. Statistics and data presentation

Results are presented as mean \pm standard error of mean (SEM) unless otherwise stated. Statistical significance was tested using unpaired students *t*-test or one-way ANOVA with Tukey's post-hoc test. $P < 0.05$ was considered statistically significant. All statistics and graphs were performed using GraphPad Prism (California, USA). Illustration and micrographs were created using Adobe Illustrator and Photoshop software.

3. Results

3.1. Body weight and hormone levels

A detailed description of the in vivo data has been published previously [31]. These data showed no significant change in either food intake or bodyweight throughout the 1.5 month duration of this study (Table 1). HbA_{1c} levels were unchanged 1.5 months following IT surgery, and only minor improvements were noted in terminal fasting plasma glucose (Table 1). Hence, no animals developed frank diabetes during the post-surgical period. Fasting insulin levels were significantly lower in the IT group (Table 1) compared to the sham-operated animals

Table 1

Body weight and plasma parameters: all measurements were obtained 1.5 month post-surgery, except GLP-1 measurements obtained at $t = 15$ min following OGTT one month post-surgery. In detail description of the vivo data is previously published [31]. All values are presented as mean \pm SEM. Statistical analysis: One-way ANOVA with Tukey's multiple comparison post-hoc test, (*Sham/IT vs. Presurgery; ^Sham vs. IT; $p < 0.05$ for significance).

	Presurgery	Sham	IT
Terminal body weight	383 \pm 6.6	560 \pm 14.9	536 \pm 6.9
Fasting blood glucose (mmol/L)	5.5 \pm 0.18	6.6 \pm 0.21 ^{*^}	5.7 \pm 0.06
HbA _{1c} (%)	4.0 \pm 0.16	4.4 \pm 0.20	3.8 \pm 0.13
Fasting insulin (ng/ml)	0.95 \pm 0.12	3.4 \pm 0.34 ^{*^}	1.7 \pm 0.23
Total GLP-1 _{OGTT} = 15' (pg/ml)	N.A.	17.6 \pm 4.2 [^]	73.2 \pm 7.5
Active GLP-1 _{OGTT} = 15' (pg/ml)	N.A.	4.2 \pm 0.5 [^]	6.7 \pm 0.6

whereas active and total OGTT GLP-1 plasma levels were significantly increased 1.5 months post-surgery (Table 1).

3.2. Gut volume and surface area

The length of the intestinal regions did not differ between groups (Table 2). Stereological quantification of total and regional gut volumes revealed a significant increase in the Jejunum and Distal ileum in the IT-operated rats compared with the sham group (Fig. 2A–B) which was related to a significant increase in both mucosa (Fig. 2C), and submucosa/muscularis/serosa volume (Fig. 2D). The greatest mean increase in mucosa volume was observed in the transposed IT segment, which was almost 70% enlarged compared with that in sham animals ($P < 0.05$) (Fig. 2C). No significant changes ($P > 0.05$) were observed in the colon (SHAM 1.76 ± 0.15 vs. IT 2.05 ± 0.13 cm³). The increased mucosal volume was associated with a similar increase in mucosal surface area, reaching statistical significance in the IT region (Fig. 2E), and with no significant changes in the colon (SHAM 89.20 ± 9.93 vs. IT 95.80 ± 7.52 cm³).

3.3. Number and density of enteroendocrine cells

Chromogranin A immunoreactive cells were distributed across the entire mucosa in both sham and IT surgery groups (Fig. 3A). A similar distribution pattern was observed for CCK and 5-HT positive cells. In contrast, GLP-2 positive cells were restricted primarily to the crypts and basal part of the villi while neurotensin positive cells were located mainly in the apical parts of the villi. In line with the increased mucosal volume, the total population of enteroendocrine chromogranin A immunoreactive cells quantified stereologically was markedly increased in the IT region compared to sham animals (Fig. 3B) from approximately 2.2 million cells to 3.5 million. In contrast, chromogranin A immunoreactive cell density was significantly reduced by approximately 20% in the IT group compared with sham (Fig. 3C). Immunohistochemical staining for CCK, GLP-2, 5-HT and neurotensin in the IT region (Fig. 4A–D) showed that all four enteroendocrine cell subtypes were to be markedly increased in number in the IT region, most noticeably the CCK and GLP-2 immunoreactive cells, respectively (Fig. 4E). The density of CCK and GLP-2 immunoreactive cells did not differ between IT and sham groups, but both neurotensin and 5-HT cell density were reduced in the IT rats (Fig. 4F).

4. Discussion

The present study aimed to investigate the short-term effect of IT surgery on intestinal morphology and EEC numbers in pre-diabetic UCD-T2DM rats. Using mathematically unbiased stereological methods we report that IT surgery led to a marked increase in volume and intraluminal surface area in the transposed and adjacent distal region of the ileum, which was coupled to an increased number of EECs. The increase in the total number of EECs, as well as in individual counts of GLP-2, CCK, 5-HT and neurotensin immunoreactive cell types, is considered a fundamental cause of the increased circulating gut hormones observed after IT surgery and the powerful effect of this kind of surgery on obesity-associated diseases, like type-2-diabetes.

Table 2

Intestinal length: length of segments at termination 1.5 months post-surgery.

Length (cm)	Sham	IT
Anterior gut	6.8 \pm 2.7	10.6 \pm 5.5
IT	21.5 \pm 5.0	21.4 \pm 1.3
Jejunum	45.9 \pm 8.0	50.9 \pm 6.0
Distal ileum	10.8 \pm 3.8	8.9 \pm 1.0
Total jejunioileum	85.0 \pm 7.4	91.8 \pm 6.1

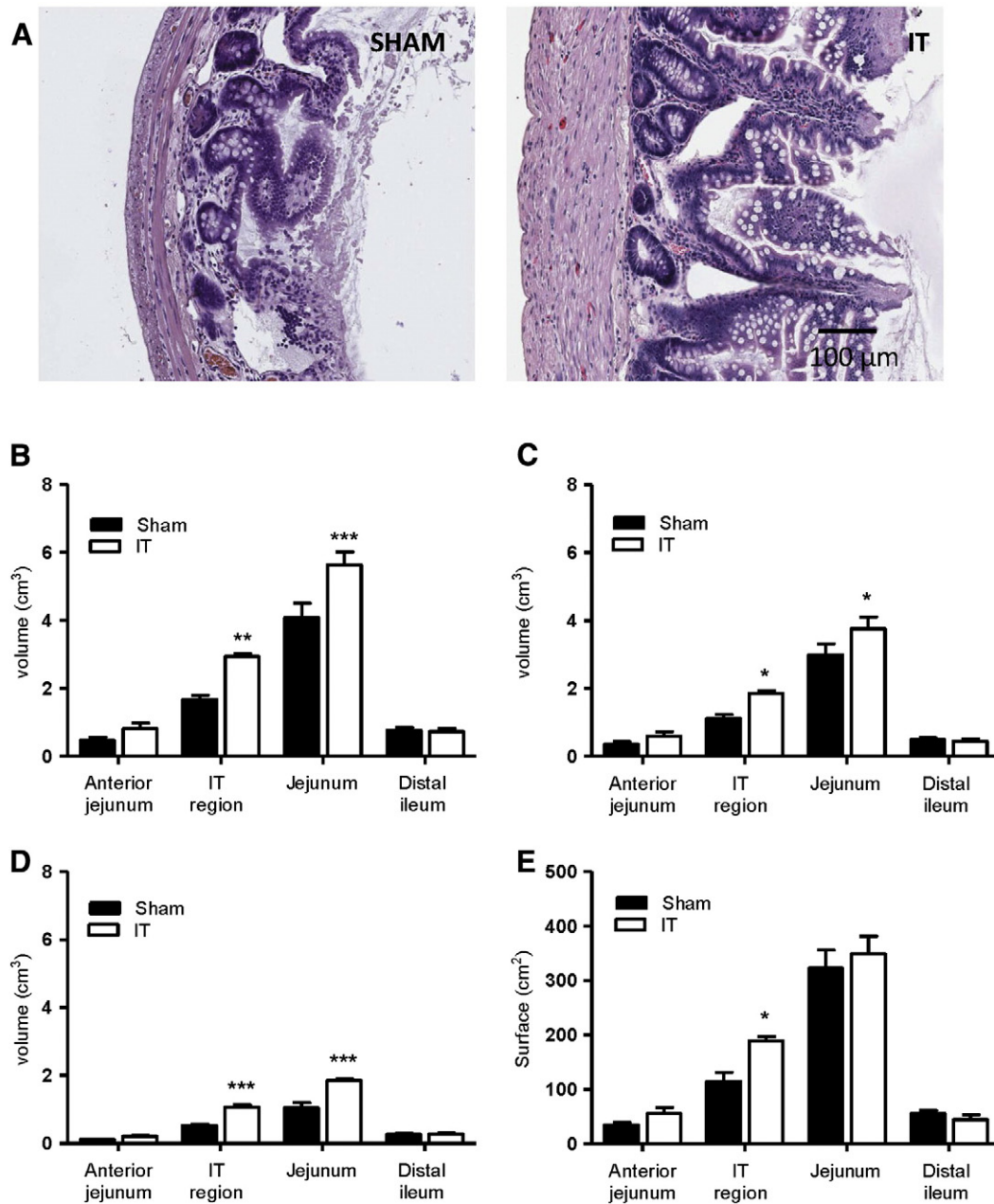


Fig. 2. Gut volume and surface area: representative images from both groups showing evident hypertrophy in the transposed region of IT animals compared with SHAM (A). Estimates of total regional volume (B), total regional mucosa volume (C), and total submucosa/muscularis/serosa volume (D). Total luminal surface area (E) (* $p < 0.05$ for significance).

The current data set represents the first stereological quantitative analysis of EEC populations after IT surgery in a rodent model of type 2 diabetes. Stereological methods have previously been shown to be superior to traditional morphometric analysis using planar measurements of villus length [37] and is distinguished by producing absolute values of intestinal volume, surface area and the number of cells instead of ratios, thereby allowing for a better comparison between studies. Our estimates of total volume and surface area in the sham group are in line with previous findings from other experiments in laboratory rats [38–41].

Previous studies have looked into the effects of IT surgery on gut morphology and specific EEC dynamics using conventional methods. The findings were coupled with changes in hormonal gene expression patterns as well as hormonal plasma levels in rodents [1,21,42–50]. Collectively, these data confirmed that IT surgery leads to considerable changes in the gut. Gut hypertrophy and cellular hyperplasia as well

as increased gut hormone gene expression and secretion are now well-known characteristics of several gastrointestinal surgical interventions, including gut resection and other types of bariatric surgery [18,51–56]. These morphological changes have been suggested to develop as compensatory mechanisms to overcome the reduced absorptive capacity [57] of the intestine. However, in the IT surgical model there is no change in gut length, nutrient absorption or food intake, suggesting that a different factor is leading to the post-operative morphological changes. In this respect, GLP-2 may be of specific interest since GLP-2 signaling increases intestinal growth by enhancing crypt cell proliferation and inhibiting apoptosis [8,9]. Like GLP-1, plasma GLP-2 is known to be highly increased in IT surgical models [50,58], and known to affect gut morphology following exogenous treatment [51,55]. Moreover, alterations in food composition and thus changes in macronutrients or bile acid concentrations may affect L-cell derived hormone release. Detection of nutrients by distal

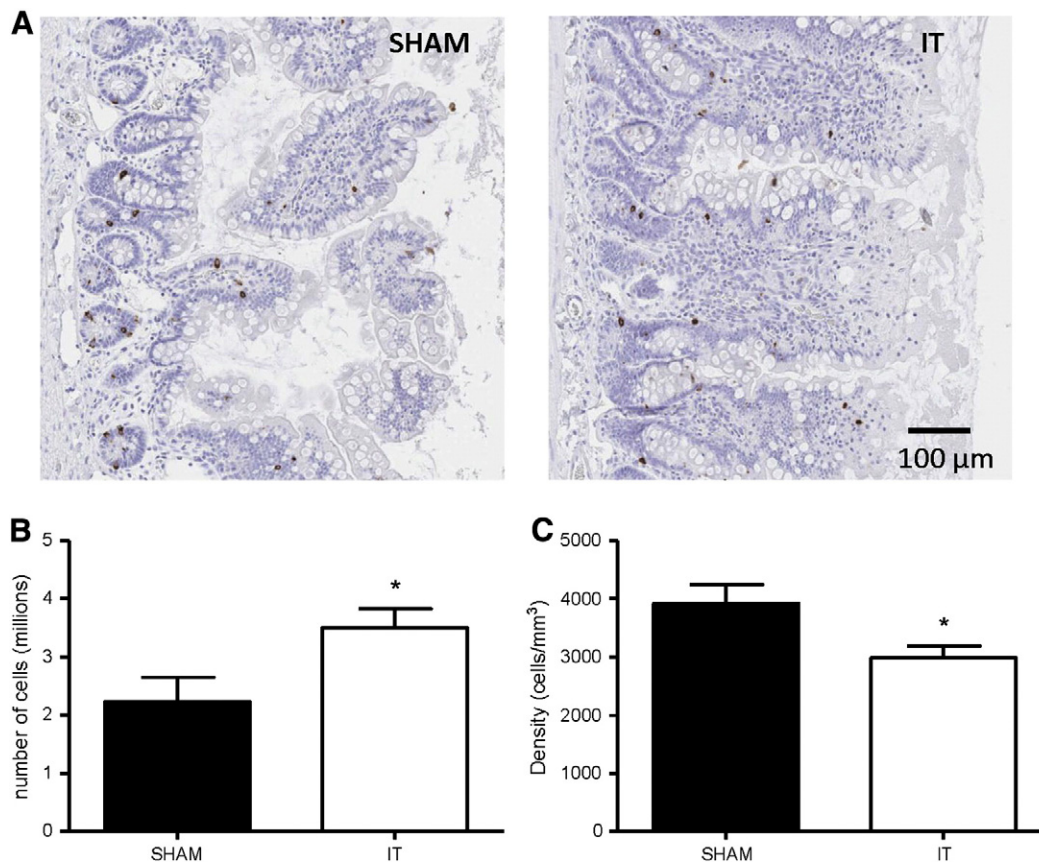


Fig. 3. Enteroendocrine cell number and density. Images from the IT region in SHAM and IT surgery groups stained for chromogranin A (A). Total number of cells (B) and density (C) of enteroendocrine chromogranin A immunoreactive cells in the IT transposed region (* $p < 0.05$ for significance).

enteroendocrine cells stimulates both GLP-1 and GLP-2 secretion [8,59]. Similarly, bile acid stimulation of the TGR5 receptor located on GLP-1 secreting cells [30,42] also affects hormone release [60,61].

The primary objective of this study was to evaluate changes in the total number of EECs in the IT region on the basis of chromogranin A immunoreactivity. In contrast to our previous studies focusing specifically on L-cell number and density in RYGB and Zucker Diabetic Fatty (ZDF) rats [15,18], we observed an unexpected change in overall EEC density in the IT region. This observation led us to further examine a number of endocrine cellular subtypes based on their hormonal content. The various roles of hormones and peptides released from the gut coupled with the known, but still not fully elucidated effect of L-cell derived peptides on food intake, body weight and glucose homeostasis, were considered important to better understand the effect of IT surgery on diabetes prevention in the UCD-T2D rat model. Serotonin (5-HT), which is produced by the enterochromaffin (EC) cells, is already known to influence gastrointestinal motility and plays important roles in food intake and control of body weight [62,63]. Similarly CCK, which is produced by the intestinal I-cells, is considered a key hormone in gut signaling of hunger and satiety [64]. Neurotensin, produced by the intestinal N-cells, is implicated in appetite regulation, stimulation of pancreatic secretion, inhibition of gastric/small bowel motility and gastric acid secretion [65]. The physiological implications of these hormones underscore the importance of gaining additional data on their numbers and density after IT surgery. To date both CCK and neurotensin levels have been reported to be increased following bariatric intervention [66,67], whereas relatively little is known about the regulation of serotonin [68].

Our quantitative analyses demonstrated a significantly increased number of all ECC subtypes in the IT region. The density of GLP-2 and

CCK immunoreactive cells was unchanged between IT and sham-operated rats, but the density of neurotensin and 5-HT immunoreactive cells was significantly reduced. Despite corroborating our previous findings on L-cell hyperplasia in ZDF and RYGB rat models [15,18] these data somewhat contradict our earlier hypothesis that all endocrine cells, which differentiate from a common pluripotent stem cell in the crypt of the intestine [69–72], are genetically encoded in the mucosal turnover and are directly correlated to changes in intestinal volume. The current data suggest that more complicated regulatory mechanisms are involved than mucosal hypertrophy alone. One should note that the sum of the four cell subtypes surpasses the total number of ECCs. This discrepancy may be related to differences in the antigenicity of the antibodies, but it may conversely also imply that the individual cells are able to express more than one hormone. GLP-1, GIP (K-cells) and CCK-positive cells have previously been shown to overlap in the upper intestine [73,74], whereas PYY, GLP-1, CCK, and neurotensin are known to be co-expressed in the same cells in the distal gut [75,76]. This challenges the traditional classification of endocrine cell types based on their hormonal content [77] and may explain the changes in cell density. Moreover, since the endocrine cells differentiate as they migrate towards the tips of the villi before they eventually undergo apoptosis and are extruded into the lumen [71,76,78], this may also explain the reduced density of the more apically located 5-HT and neurotensin-labeled cells. The density of neurotensin-labeled cells is also significantly reduced in rats following RYGB, whereas the densities of GLP-1, CCK and 5-HT are not [54]. There is presently no adequate explanation for the discrepancy between Mumphy's data showing no changes in the density of 5-HT, and our data showing a decrease, but it may be related to the different models used, the different segment analyses and importantly the different methodology.

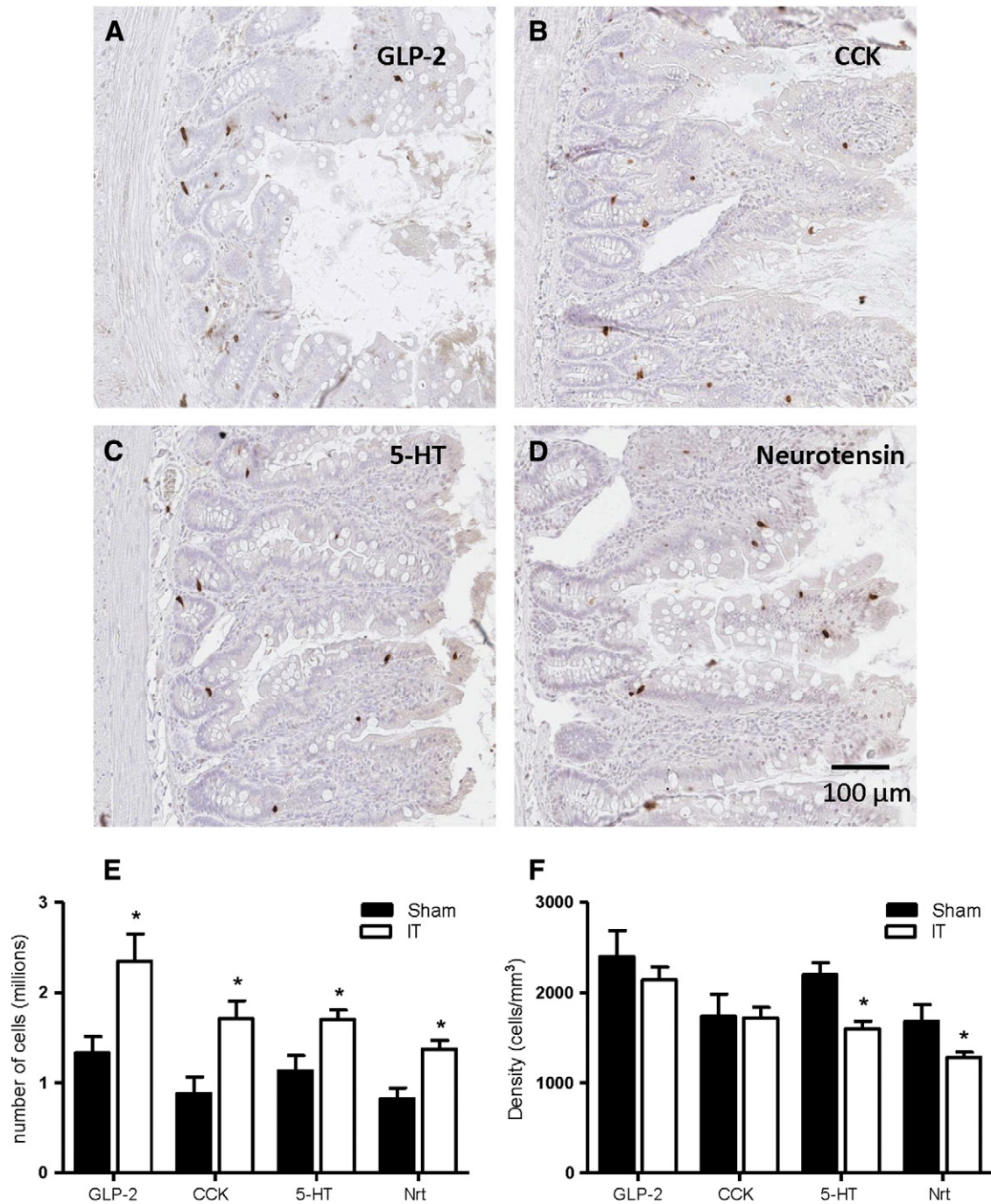


Fig. 4. Enteroendocrine cell subtypes. Images from the IT region stained for GLP-2 (A), CCK (B), 5-HT (C) and neurotensin (D) immunoreactivity. The total number (E) and density (F) of CCK, GLP-2, neurotensin and 5-HT cells in the IT transposed region (* $p < 0.05$ for significance).

In the present study the trophic effects on the gut was most predominant in the transposed region, with less effects in the remaining jejunum. The same pattern was observed by Thulesen et al. [58] examining differences in tissue GLP-2 and plasma GLP-2 levels following ileal–jejunal transposition surgery. GLP-2 is thought to have both endocrine and paracrine effects [79]. Thus the indirect paracrine effect of GLP-2 could potentially explain the greater trophic effect observed in the hyperstimulated transposed region. Collectively, our study confirms and extends the reports of gut hypertrophy and EEC hyperplasia after various types of gut surgeries.

In summary, the current study demonstrates that IT surgery leads to a rapid intestinal hypertrophy that is independent of body weight and

diabetic state. The marked hypertrophy is coupled with a significant increase in the total number of ECCs in the transposed region potentially contributing to an increased post-prandial hormonal secretory capacity and the marked effect of IT surgery on diabetes prevention in the UCD-T2D rat.

Conflict of interest statement

CFH and EV are currently employed by Gubra. NV and JJ are main shareholders of Gubra. PSA and BPC have no conflict of interest. PH has nothing to declare.

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